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Auditory Evoked Potentials Dynamically Related to Sleep-Waking States in Unrestrained Rats

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ABSTRACT—Auditory evoked potentials (AEPs) to continuous click stimuli delivered at 1-s intervals were bipolarly recorded between the frontal and the fronto-parietal cortex in freely behaving rats throughout the first 3 hours of the light and the dark period. Dynamic changes in the middle and late latency components of AEPs were serially analyzed during slow wave sleep (SWS), paradoxical sleep (PS) and wakefulness (W). The sleep-waking stages affected greatly the latency of the first and second negative (N_1 and N_2) and positive (P_1 and P_2) waves. Especially during SWS, N_2 and P_2 dynamically changed: the deeper SWS, as evidenced by an elevated delta activity, was accompanied by the longer latency and the higher amplitude. The peak-to-peak voltage difference was maximal when delta-sleep occurred. During PS, AEPs remained quite stable, exhibiting a steady level of N_2 and P_2 amplitudes and no fluctuation of their latencies. During W, N_2 tended to decrease in amplitude and sometimes disappeared due to habituation to the stimuli. Significant circadian variations were found in the latency and amplitude of the middle and late AEP components. Thus, the state-dependent and time-of-day-dependent characteristics of AEPs might be utilized as a good indicator for sleep-vigilance scoring.

INTRODUCTION

An electroencephalogram (EEG) is universally adopted as an objective index for scoring the sleep-waking stages, which requires time-consuming analysis and a large storage space. Auditory evoked potentials (AEPs) can also provide an objective evaluation of the sleep-waking state in a more concise form. A definite change in the latency and/or amplitude of AEPs is known to occur during sleep-waking cycles in humans [1–11], cats [12–16] and rats [17, 18]. In the late components of AEPs, markedly increased amplitudes are observable during slow wave sleep (SWS) in animals or non-REM sleep in humans, although the early components equivalent to brainstem evoked responses are considerably stable in humans [6, 19]. However, in these studies, attention was mainly focussed on the analysis and comparison of AEPs at a certain steady state of sleep-waking stages.

Since the conscious level of wakefulness (W) and the stages of sleep dynamically change as a function of time, a continuous time-course analysis of AEPs is of special interest. As far as the present authors know, Molnár *et al.* [15] noted that the peak latency of the latest negative component of AEPs shifts forward during the rapid eye movement phase of paradoxical sleep (PS) in cats; whereas Ujszászi and Halász [8] observed that the latency and amplitude of late components of AEPs show a considerable variety during stage 2 non-REM sleep in humans. The present paper deals with the first systematic approach to a dynamic aspect of AEP variations, both minute-to-minute and day-to-night, with special reference to the

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sleep-waking stages, i.e. SWS, PS and W, in freely behaving rats.

Since the rat is a multiphasic sleeper and a night-active animal, approximately two thirds of total sleep time are distributed in the light period under a 12-h light and 12-h dark schedule [20]. The duration of SWS and PS episodes is longer in the light period than in the dark period, while that of W episodes is shorter in the light period [21]. Hence the question arises as to whether circadian variation in the AEP components exist between the early phases of the light and the dark period. The experimental facts dealt with here indicate that this is really the case. The preliminary results are published elsewhere in abstract form [22–24].

MATERIALS AND METHODS

Seventeen male rats of the Sprague-Dawley strain, raised in our closed colony on a 12-h light and 12-h dark schedule (light period: 08:00–20:00 h) under a constant air-conditioned environment of $25\pm1^{\circ}C$ and $60\pm6\%$ relative humidity with free access to rat chow and water, were used. At the age of 60–90 days, animals weighing 300–450 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.), placed on a stereotaxic apparatus and permanently implanted with three cortical electrodes for EEG and AEP recording, two nuchal electrodes for electromyogram (EMG) recording, and a silver plate on the skull as a reference electrode. The EEG-AEP electrodes were located on the surface of the frontal cortex (1.8 mm lateral to the central suture and 4.5 mm anterior to the bregma) and of the fronto-parietal cortex (3.7 mm lateral and 1.5 mm posterior, as above). EEG and AEP were bipolarly recorded between the two electrodes. The surgical procedure was the same as described in a previous paper [25].

The rats were individually housed in a special cylindrical cage which enabled continuous monitoring of EEG and EMG, and continuous auditory stimulation (Fig. 1). A slip-ring fixed above the cage guaranteed the free movement of the rats. Each cage was placed in a sound-proof, electromagnetically shielded chamber under the same environmental conditions as above. A week was

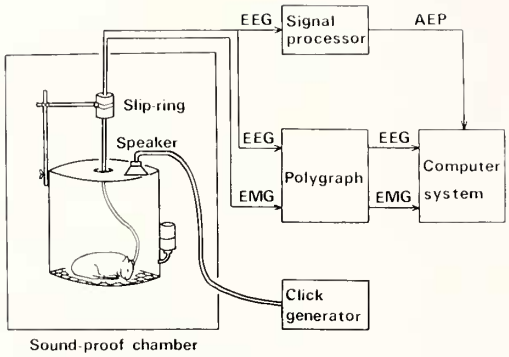

FIG. 1. Experimetal system. For details, see text.

allowed for recovery from surgery before the experiment. Then EEG and EMG were polygraphically recorded, and SWS, PS and W were visually scored according to the routinized criteria [20]. After observing a steady circadian rhythmicity in sleep-waking amounts, auditory stimuli consisting of clicks were delivered at 1-s intervals through a speaker placed above the cage. The clicks were 0.1-ms square wave pulses, the intensity of which was adjusted to 70 dB above the noise level on the floor of the cage. Click stimuli were continuously given to the rats either between 08:00 h and 11:00 h or between 20:00 h and 23:00 h. Under continuous recordings of EEG and EMG, AEPs were collected from two out of the three cortical electrodes for averaging. Fifty AEPs were averaged at one time by a signal processor (7T17, NEC San-ei), stored in a floppy disk and simultaneously recorded on a plotter in the following 10 seconds. Hence each averaged AEP was recorded at 1-min intervals. Averaged AEPs were then analyzed with reference to the EEG-EMG defined sleep-waking stages and to the delta activity (0.5–3.5 Hz) of the EEG records which was filtered and integrated at 1-min intervals. For an analysis of circadian variations, AEPs recorded at the definite occurrence of SWS, PS and W in the 1-h period of either 08:00–09:00 h or 20:00–21:00 h were respectively compared and statistically analyzed by Student's *t*-test.

RESULTS

AEP components

Typical AEPs during SWS, PS and W in freely

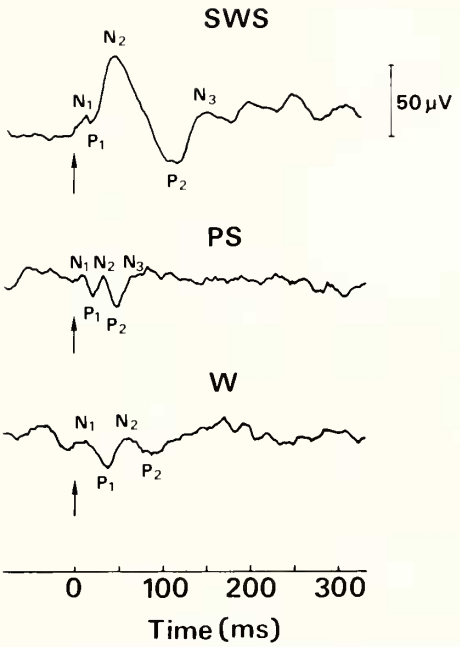

FIG. 2. A typical example of averaged AEPs with the definition of peaks during SWS, PS and W. The arrow indicates click stimuli.

behaving rats are shown in Figure 2. The waveforms were largely in accordance with those described in previous studies [17, 18]. The middle and late components of AEPs were composed of several positive and negative deflections in their waveforms. The peaks were designated as the first negative (N_1) and positive (P_1) waves, the second negative (N_2) and positive (P_2) waves, and so forth, according to their polarity and their sequence order. The waveform, the latency of peaks, and the peak-to-peak amplitude changed dynamically and were largely dependent on sleep-waking stages, as described below.

AEPs during SWS

In the typical waveform of an AEP during SWS, the first peaks, N_1 and P_1, clearly appeared within 30 ms after the onset of click stimuli. The N_1–P_1 amplitude was considerably small, never exceeding 10 μV. Large N_2 and P_2 deflections then followed around 50 ms and 120 ms after the onset of click stimuli, respectively. Hence the difference between these peaks became very large, sometimes exceeding 80 μV, which was far greater than that during PS and W. Subsequent peaks (N_3, P_3, N_4 and P_4) were observable in a latency range from 110 to 200 ms (Fig. 2). The waveform of AEPs varied dynamically during the course of SWS (Fig. 3, right). Deep SWS, as evidenced by the elevated occurrence of delta activity, was characterized by a prolongation of N_2 and P_2 latencies and a profound increase in their amplitude. In contrast, the N_2 latency and amplitude declined in accordance with the reduction of delta activity (Fig. 3, left).

AEPs during PS

During PS, definite rises and falls in amplitude occurred three times within 100 ms after click stimuli (N_1–N_3 and P_1–P_3, Fig. 2), and some rats exhibited 4th deflections (N_4 and P_4). All peak-to-peak amplitudes were smaller than those of SWS, never exceeding 30 μV. Wave components were not clearly distinguishable later than 100 ms after stimulation. AEP components were characterized by their stability during the course of PS (Fig. 4). The latency of N_2 and P_2 showed little fluctuation. The N_2–P_2 amplitude remained at a steady level. Sometimes, the duration of PS episodes was too short for a time-series analysis of successive AEPs, which were averaged at 1-min intervals.

AEPs during W

In the waking state, N_1 and P_1 appeared approximately 20 ms and 40 ms after click stimuli, respectively (Fig. 2). Subsequent wave components, N_2 and P_2, occurred in the following 50 ms. No clear waveform was observable after then. Peak-to-peak amplitudes varied considerably, ranging from 5 to 40 μV. The N_2 and P_2 tended to decrease their amplitude and often almost disappeared (Fig. 5, right). Television monitoring revealed that, during such a change, rats sometimes displayed definite behavior such as eating, drinking and grooming. N_2 and P_2 waves usually reappeared during the course of the same W episode. The N_1 and P_1 latency was relatively stable (Fig. 5).

Time-of-day-dependent variation in AEPs

AEPs in the early phase of the light period were compared to those of the dark period. Since

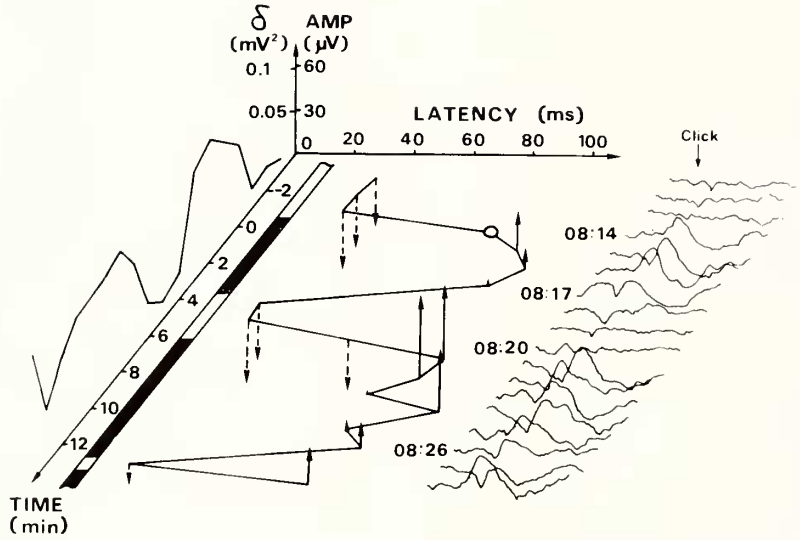

FIG. 3. A typical example of dynamic changes in the amplitude and latency of N_2 component of AEPs during SWS. The corresponding AEPs are shown at the right side. AMP (ordinate) means the difference between the reference N_2 amplitude at the initial SWS (indicated by an open circle) and that of the other AEPs. Increments and decrements in absolute values are expressed by arrows directed upward and downward, respectively. Sleep-waking stages are shown by an oblique column, in which SWS, PS (shown only in Fig. 4) and W are reprcsented by black, dotted and white sections, respectively. The initial and final time of day is indicated for the main episodes. Integrated delta activity (δ) is shown at the left side.

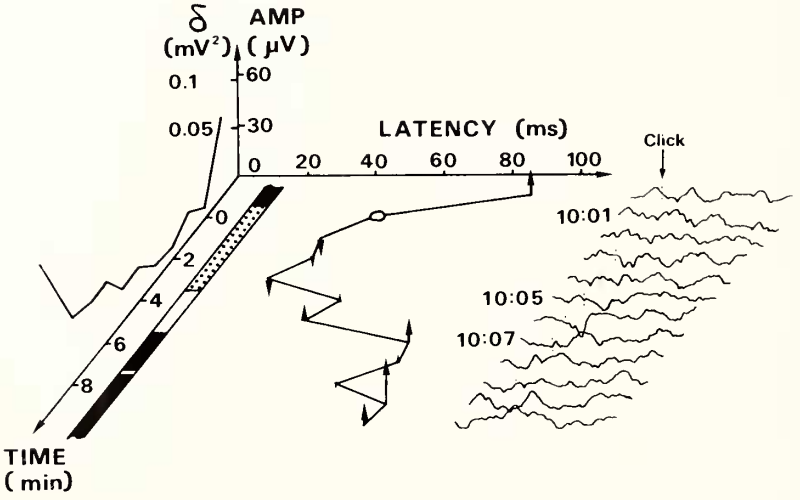

FIG. 4. A typical example of dynamic changes in the amplitude and latency of N_2 component of AEPs during PS. AMP (ordinate) means the difference between the reference N_2 amplitude at the initial PS (indicated by an open circle) and that of the other AEPs. For further explanations, see the legend of Fig. 3.

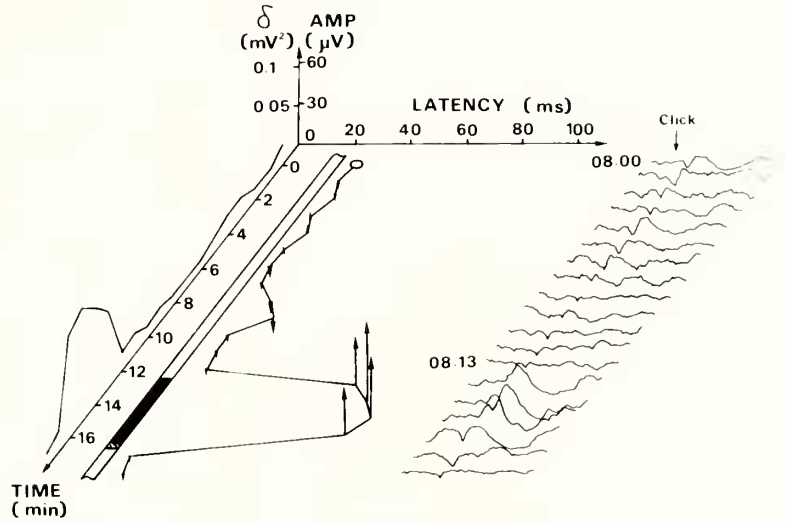

FIG. 5. A typical example of dynamic changes in the amplitude and latency of the P_2 component of AEPs during W. AMP (ordinate) means the difference between the reference P_2 amplitude at the initial W (indicated by open circle) and that of the other AEPs. For further explanations, see the legend of Fig. 3.

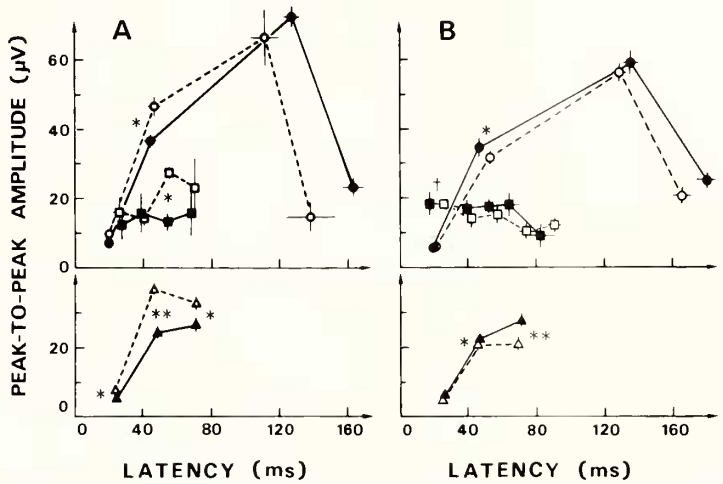

FIG. 6. The peak-to-peak amplitude and latency of AEP components as a function of the light-dark period and sleep-waking stages in two different rats A and B. Values are mean±SEM during the early light period (08:00–09:00 h, A: n=10 for SWS; n=3 for PS; n=24 for W, B: n=18 for SWS; n=4 for PS; n=7 for W) and the early dark period (20:00–21:00 h, A: n=16 for SWS; n=3 for PS; n=18 for W, B: n=11 for SWS; n=3 for PS; n=15 for W). Circles, squares and triangles indicate respectively SWS, PS and W, in which white and black signs mean respectively the light and the dark period. Peak-to-peak amplitudes are expressed in absolute values and arrranged in sequence from left to right: the N_1–P_1 amplitude, the P_1–N_2 amplitude, the N_2–P_2 amplitude and the P_2–N_3 amplitude (shown only for PS in rat A). Asterisks indicate that the peak-to-peak amplitude difference between the light and the dark period was statistically significant at $P<0.05$ (*) and $P<0.01$ (**). A symbol (†) indicates that the latency difference between the light and the dark period was statistically significant at $P<0.05$.

individual variations were so large, no clear difference was found in average values from the results pooled for all animals. However, if data were individually processed for all AEPs during each state, a significant circadian variation was obtained from most of the rats. Figure 6 illustrates typical examples, in which the peak-to-peak amplitude and latency of AEP components showed significant differences between the light and the dark period.

During SWS, the differences in each peak-to-peak amplitude were apparent. In most cases, the P_1–N_2 amplitude significantly differed between the two periods. The voltage difference ranged from 3 to 10 μV. The later components showed usually a larger difference which was insignificant due to considerable variations. The latency of P_2 and N_3 during nocturnal SWS was largely delayed by 5–20 ms in comparison with that during diurnal SWS. However, no statistical significance was detected because of large variations.

During PS, the circadian difference in the AEP parameters was rather small and statistically insignificant except for that of some components (for examples, Fig. 6A: the N_2–P_2 amplitude significantly differed by 14 μV; Fig. 6B: the N_1 latency significantly differed by 8 ms).

During W, the peak-to-peak amplitude of most AEP components significantly differed between the light and dark periods. The difference ranged from 2 to 15 μV. However, their latency exhibited little difference.

DISCUSSION

The middle and late components of AEPs in freely behaving rats exhibited state-dependent changes. It was found that minute-to-minute variations occurred in the waveform of AEPs during the course of W. In addition, our time series analysis first demonstrated that the latency and amplitude of the AEP components, especially during SWS, varied dynamically with close relation to the fluctuations in the EEG delta activity. Since the delta activity is regarded as a reliable indicator of deep sleep, it is likely that the AEP parameters might specifically indicate the time-course changes in the state of sleep-wakefulness.

The AEP activity in our rats was most prominent during the deep sleep stage. This was comparable to the previous reports in which auditory stimuli of a low frequency, up to 10 Hz, were given to cats [12–16] and rats [17,18]. It seems likely that in these animals, auditory inputs at a relatively low frequency may easily provoke a larger amplitude and a longer latency in the middle and late AEP components of AEPs during SWS than during PS and W.

The mechanism involved in this change remains unknown. There are several speculations. Firstly, Weitzman and Kremen [8] concluded that AEPs during sleep represents summed K complexes elicited from the auditory stimulation. Secondly, the chronic twitches of the middle ear muscles during PS are responsible for a reduction of auditory inputs, which eventually causes a reduction in the amplitude of AEPs [12, 13]. This assumption, however, is not confirmed by a later study [17]. Thirdly, a state-dependent change in body temperature may be considered. It is reported that heating and cooling of the body can respectively shorten and enlarge the latency and amplitude of auditory brainstem responses [26]. Since the early phase of an SWS episode generally accompanies a fall in body temperature [27], the slower middle and late latencies of AEPs during SWS might be accounted for by the lowered temperature, which accompanies a delayed synaptic transmission. Finally, the blockade of sensory information in neural circuits during W may be considered. Attention causes changes in the latency of late components of AEPs [28]. This was largely due to the habituation provoked by the inhibitory mechanism or the gating to the monotonous stimulation. Therefore, changes in attentive levels might reflect the amplitude fluctuations. In contrast, during SWS no attentive activity exists and the slow wave generator mechanism is predominant. This may result in an enlarged AEP waveform. At present no information is available to determine which possibility is most plausible.

In human studies, however, the results are conflicting; some investigators report a similar increase in peak amplitudes and a prolongation of their latencies in deep sleep stages [6, 8–11], whereas others report little changes depending on

the sleep-waking stages (see [29]); most authors refer to a reduced AEP activity during PS, while Ornitz *et al.* [5] demonstrated an enlarged waveform during PS. Furthermore, if auditory stimuli are given at sufficiently high frequency, up to 60 Hz, the amplitude changes are smaller during sleep than during waking [3, 4].

The present study first detected the diurnal and nocturnal differences in the amplitude and latency of the middle and late AEP components. In this connection, Hanada and Kawamura [30] noted that a clear circadian variation occurs in the amplitude of an early component of evoked potentials caused by electrical stimulation of the optic tract in rats. These facts may indicate the existence of a time-of-day-dependent change in the sleep-waking state. However, the existence of large individual variations in these AEP parameters may indicate that the differences between the light and dark periods reflected not only the rhythmicity derived from the circadian oscillator but also the variations in the behavioral situations in each rat, since an attentive behavior during W or an elevated delta activity during SWS might easily modify the AEP parameters.

Apart from the above discussions, the close correlation between the AEP parameters and the sleep-waking stages, especially during SWS, may suggest that the time-consuming sleep scoring based on polysomnography could be replaced or supplemented by the time-course analysis of AEPs. The latter technique seems to be simpler, more conventional and easier to define the dynamic state changes. Hence the AEP dynamics could be applied not only to the evaluation of sleepiness [31] but also to an objective sleep-vigilance scoring.

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REFERENCES

1 Buchsbaum, M., Gillin, J. C. and Pfefferbaum, A. (1975) Effect of sleep stage and stimulus intensity on auditory evoked responses. Psychophysiology, **12**: 707–712.

2 Erwin, R. and Buchwald, J. S. (1986) Midlatency auditory evoked responses: differential effects of sleep in the human. Electroencephalogr. Clin. Neurophysiol., **65**: 383–392.

3 Linden, R. D., Campbell, K. B., Hamel, G. and Picton, T. W. (1985) Human auditory steady state evoked potentials during sleep. Ear Hearing, **6**: 167–174.

4 Linden, R. D., Picton, T. W., Campbell, K. B. and Hamel, G. (1985) Thresholds for auditory steady state evoked potentials during sleep. In "Sleep '84". Ed. by W. P. Koella, E. Rüther and H. Schulz, Gustav Fischer Verlag, Stuttgart, pp. 228–231.

5 Ornitz, E. M., Ritvo, E. R., Carr, E. M., Paniman, L. M. and Walter, R. D. (1967) The variablity of the auditory evoked response during sleep and dreaming in children and adults. Electroencephalogr. Clin. Neurophysiol., **22**: 514–524.

6 Osterhammel, P. A., Shallop, J. K. and Terkildsen, K. (1985) The effect of sleep on the auditory brainstem response (ABR) and the middle latency response (MLR). Scand. Audiol., **14**: 47–50.

7 Ujszászi, J. and Halász, P. (1986) Late component variants of single auditory evoked responses during NREM sleep stage 2 in man. Electroencephalogr. Clin. Neurophysiol., **64**: 260–268.

8 Weitzam, E. D. and Kremen, H. (1965) Auditory evoked responses during different stages of sleep in man. Electroencephalogr. Clin. Neurophysiol., **18**: 65–70.

9 Williams, H. L., Marlock, H. C., Marlock, J. V. and Lubin, A. (1964) Auditory evoked responses and EEG stages of sleep. Ann. New York Acad. Sci., **112**: 172–179.

10 Williams, H. L., Tepas, D. I. and Marlock, H. C., Jr. (1962) Evoked responses to clicks and electroencephalographic stages of sleep in man. Science, **138**: 685–686.

11 Yokoyama, T., Ryu, H., Uemura, K., Miyamoto, T., Yamamoto, T., Furuya, Y. and Nakajima, S. (1986) Middle latency auditory evoked potentials in awake and asleep. Rinsho Noha, **28**: 9–44.

12 Baust, W., Berlucchi, G. and Moruzzi, G. (1964) Changes in the auditory input in wakefulness and during the synchronized and desynchronized stages of sleep. Arch. Ital. Biol., **102**: 657–674.

13 Berlucchi, G., Munson, J. B. and Rizzolatti, G. (1967) Changes in click-evoked responses in the auditory system and the cerebellum of free-moving cats during sleep and waking. Arch. Ital. Biol., **105**: 118–135.

14 Chen, B. M. and Buchwald, J. S. (1986) Midlatency anditory evoked responses: differential effects of sleep in the cat. Electroencephalogr. Clin. Neurophysiol., **65**: 373–382.

15 Molnár, M., Kormos, G. and Csépe, V. (1986) Laminar analysis of intracortical auditory evoked potentials during the wakefulness-sleep cycle in the cat. Int. J. Psychophysiol., **3**: 171–182.

16 Winters, W. D. (1964) Comparison of the average cortical and subcortical evoked response to clicks during various stages of wakefulness, slow wave sleep and rhombencephalic sleep. Electroencephalogr. Clin. Neurophysiol., **17**: 234–245.

17 Hall, R. D. and Borbély, A. A. (1970) Acoustically evoked potentials in the rat during sleep and waking. Exp. Brain Res., **11**: 93–110.

18 Knight, R. T., Brailosky, S., Scabini, D. and Simpson, G. V. (1985) Surface auditory evoked potentials in the unrestrained rat: component definition. Electroencephalogr. Clin. Neurophysiol., **61**: 430–439.

19 Campbell, K. B. and Bartoli, E. A. (1986) Human auditory evoked potentials during natural sleep: the early components. Electroencephalogr. Clin. Neurophysiol., **65**: 142–149.

20 Honda, K. and Inoué, S. (1981) Effects of sleep-promoting substance on sleep-waking patterns of male rats. Reports Inst. Med. Dent. Eng., **15**: 115–123.

21 Honda, K., Komoda, Y., Nishida, S., Nagasaki, H., Higashi, A., Uchizono, K. and Inoué, S. (1984) Uridine as an active component of sleep-promoting substance: its effects on the nocturnal sleep in rats. Neurosci. Res., **1**: 243–252.

22 David, E., Inoué, S., Okano, Y., Honda, K. and Pfotenhauer, M. (1986) Click-evozierte Potentiale im Schlaf- und Wachzustand; ein Vergleich zwischen Ratte und Mensch. Biomed. Tech., **31**: suppl. 146.

23 Inoué, S., Okano, Y., Honda, K. and David, E. (1986) Auditory evoked potentials specific to sleep-waking stages in unrestrained rats. Abstracts of 3rd Int. Evoked Potentials Symp., p. 125.

24 Okano, Y., Honda, K., David, E. and Inoué, S. (1986) Cortical auditory evoked potentials specific to the sleep-waking stages in unrestrained rats. Abstracts of lst Int. Congr. Neuroethol., p. 71.

25 Honda, K. and Inoué, S. (1978) Establishment of a bioassay method for the sleep-promoting substance. Reports Inst. Med. Dent. Eng., **12**: 81–85.

26 Marsch, R. R., Yamane, H. and Potsic, W. P. (1984) Auditory brain-stem response and temperature: relationship in the guinea pig. Electroencephalogr. Clin. Neurophysiol., **57**: 289–293.

27 Obál, F., Jr. (1984) Thermoregulation and sleep. Exp. Brain Res., Suppl. **8**: 157–172.

28 Polich, J. (1986) Attention, probability, and task demands as determinants of P300 latency from auditory stimuli. Electroencephalogr. Clin. Neurophysiol., **63**: 251–259.

29 Mendel, M. I. (1980) Clinical use of primary cortical responses. Audiology, **19**: 1–15.

30 Hanada, Y. and Kawamura, H. (1984) Circadian rhythms in synaptic excitability of the dorsal lateral geniculate nucleus in the rat. Int. J. Neurosci., **22**: 253–262.

31 Broughton, R. (1982) Performance and evoked potential measures of various states of daytime sleepiness. Sleep, **5**: 135–146.